

## Antibodies to Lipooligosaccharide of a Brazilian Purpuric Fever Isolate of *Haemophilus influenzae* Biogroup aegyptius Lack Bactericidal and Protective Activity

VICKI B. PETERS† AND LORRY G. RUBIN\*

Division of Infectious Diseases, Department of Pediatrics, Schneider Children's Hospital of Long Island Jewish Medical Center, Long Island Campus for the Albert Einstein College of Medicine, New Hyde Park, New York 11042

Received 11 February 1992/Accepted 15 May 1992

The immunological basis for protection against Brazilian purpuric fever (BPF), a fulminant infection of young children associated with bacteremia with *Haemophilus influenzae* biogroup aegyptius, is unknown. Candidate antigens to which protective antibodies may be directed include cell surface proteins and lipooligosaccharide (LOS). We studied the activity of antisera to LOS purified from a BPF *H. influenzae* biogroup aegyptius isolate. Anti-LOS antisera contained anti-LOS antibody by enzyme immunoassay and immunoblot and no detectable anti-outer membrane protein antibodies by immunoblot. Anti-LOS antisera had minimal bactericidal activity and were not protective against the homologous strain in an infant rat model of bacteremia. Antiserum to whole bacterial cells had a titer of anti-LOS antibody similar to that of anti-LOS antisera and was bactericidal and protective. Removal of anti-LOS antibodies from anti-whole cell antiserum by affinity chromatography did not result in a loss of bactericidal activity. Serum from a normal adult contained anti-LOS antibodies and had bactericidal activity. However, anti-LOS antibodies purified from this serum did not have detectable bactericidal activity. These studies suggest that anti-LOS antibodies produced in rats are not bactericidal and do not contribute to protection against experimental bacteremia with BPF strains of *H. influenzae* biogroup aegyptius.

Brazilian purpuric fever (BPF), a newly recognized fulminant infection affecting young children, is due to septicemia with particular strains of *Haemophilus influenzae* biogroup aegyptius (4). BPF is preceded by an episode of purulent conjunctivitis and is characterized by an abrupt onset of fever followed by the development of petechiae, purpura, and shock. It is often fatal. Most cases have been seen in Brazil. A single *Haemophilus influenzae* biogroup aegyptius clone designated the BPF clone is responsible for all of the cases of BPF analyzed in Brazil.

All cases of BPF have occurred in children 3 months to 10 years of age. Thus, newborns, adolescents, and adults appear to be immune. It is likely that protection against BPF is mediated, at least in part, by antibodies which are acquired by transplacental passage or in an age-related fashion. Candidate bacterial antigens against which protective antibodies may be directed include cell surface-exposed antigens such as surface proteins and lipooligosaccharide (LOS).

We have developed a model of bacteremia with BPF strains of *H. influenzae* biogroup aegyptius (BPF-Hae) in infant rats (8). Although this model does not simulate human disease, passive immunization of rats with antiserum to whole BPF-Hae cells protects against experimental bacteremia (8). In this study, we examined the bactericidal and protective activity of anti-LOS antibodies prepared against a BPF-Hae isolate.

### MATERIALS AND METHODS

**Bacteria.** BPF-Hae F3037, a blood isolate from a Brazilian child with BPF, was selected for these studies because it exhibited more virulence in rats than did other BPF-Hae isolates. This strain was animal passaged by intraperitoneal injection of rats and recovered by culture of blood. The animal-passaged strain was used in all experiments. Animal passage enhanced the virulence and was associated with a change in the LOS phenotype, as evidenced by reactivity with a panel of monoclonal anti-LOS antibodies (9). This phenotype remained stable upon repeated animal passages (7a). Bacteria were subcultured on chocolate agar plates and were grown in supplemented brain heart infusion broth to mid-logarithmic phase as previously described (8).

**Preparation and characterization of LOS.** The cells from 3 to 6 liters of logarithmic-phase growth were harvested by centrifugation. LOS was prepared by a modification of the hot phenol-water extraction procedure described by Erwin et al. to purify LOS because these authors showed that this method resulted in a more complete preparation of LOS from *H. influenzae* biogroup aegyptius than did the phenol-chloroform-petroleum ether method (2). Bacterial cells were not subjected to lysozyme and nuclease treatment prior to hot phenol-water extraction. We confirmed the findings of Erwin et al. that LOS fractionated exclusively to the phenol phase after hot phenol-water extraction (2). Crude LOS was then precipitated by addition of 0.5 M NaCl and 95% ethanol (-20°C, 18 h). The resultant pellet was suspended in distilled water, dialyzed exhaustively against water to remove phenol, and precipitated with NaCl-ethanol. The resultant crude LOS was purified by a modification of the method of Hitchcock and Brown (5). Crude LOS was solubilized in a buffer composed of 1% sodium dodecyl sulfate (SDS), 2% 2-mercaptoethanol, and 5% glycerol, and 0.5 M Tris, pH 6.8,

\* Corresponding author.

† Present address: Mount Sinai School of Medicine, New York, NY 10029.

was heated to 100°C for 10 min and treated with proteinase K (final concentration, 0.25 mg/ml; Sigma Chemical Co., St. Louis, Mo.) for 90 min at 70°C. Following reprecipitation with NaCl-ethanol and suspension in water, multiple ultracentrifugations were performed at 100,000 × *g* until the supernatant no longer contained detectable nucleic acid or protein as determined spectrophotometrically (optical densities [ODs] at 260 and 280 nm, respectively). LOS content was estimated by a *Limulus* lysate gelation assay with comparison with reconstituted U.S. standard *Escherichia coli* standard 0113 (Associates of Cape Cod, Inc., Woods Hole, Mass.). The yield of purified LOS (endotoxin) was approximately 2 mg/liter of bacteria. LOS was subjected to SDS-urea-polyacrylamide gel electrophoresis (SDS-urea-PAGE), using a 1.5-mm 7.8% (wt/vol) polyacrylamide stacking gel and 17.6% polyacrylamide separating gel, each containing 4 M urea. The samples were stacked under a constant current of 15 mA per gel until they migrated into the resolving gel; the current was then increased to 30 mA per gel until the tracking dye was approximately 1 cm from the bottom of the gel, usually in 5 to 6 h (total time). LOS bands were visualized by silver stain (Bio-Rad, Richmond, Calif.) as one or two bands of an apparent molecular mass less than that of the 14,000-Da molecular weight protein marker and greater than that of LOS from the J5 mutant of *E. coli* (2). No protein bands were visualized after silver staining or after staining of the gel with Coomassie brilliant blue.

**Production of antiserum.** Antisera were prepared in adult rats. Blood was obtained by cardiac puncture prior to immunization to provide preimmunization normal rat serum. Adult female Sprague-Dawley rats were immunized with three or four weekly injections of 25 to 100 µg of purified LOS in Freund's adjuvant. Anti-LOS serum was obtained 1 week following the third dose of LOS (immunoglobulin M [IgM] anti-LOS) or 1 to 2 weeks following a fourth dose of LOS (IgG anti-LOS). As shown in Results, the former immunization regimen contained predominantly IgM anti-LOS antibody and the latter schedule contained predominantly IgG anti-LOS antibody. Antiserum to whole organisms was obtained 1 week following three weekly injections of a suspension of whole bacteria from overnight growth on a chocolate agar plate as previously described (8). Sera were stored at -70°C.

**Preparation of affinity gels and depletion and purification of anti-LOS antibody from serum.** Two milligrams of purified LOS was coupled to 0.5 g of cyanogen bromide-activated Sepharose 4B as described by Shenep et al. (10). A control gel was prepared in an identical manner except that coupling buffer was substituted for LOS in coupling buffer.

To deplete (or sham deplete) rat antisera of anti-LOS antibodies, the gels were equilibrated with 0.01 M phosphate-buffered saline, pH 7.0 (PBS), and incubated with 1 ml of a 1:2 dilution of anti-whole cell antiserum overnight at 4°C on a test tube rotator platform. Adsorbed antiserum was recovered by centrifugation.

To prepare human anti-LOS antibodies (or a control preparation), 5 ml of human serum (from a healthy adult) was diluted 1:2 in PBS and incubated with the LOS affinity column (or a control column) by recirculating the serum over the column, using a peristaltic pump for 1 h at ambient temperature. Following removal of serum by draining the column and washing with 250 ml of PBS, bound antibodies were eluted from the column by a 5-min, ambient temperature incubation with 1 ml of 0.1 M glycine buffer, pH 2.8. The recovered material was immediately neutralized by

addition of 0.05 ml of 1 M Tris-HCl, pH 9.5, and dialyzed against PBS.

**EIAs.** Enzyme-linked immunosorbent assays (EIAs) were performed by a method described by Shenep et al. (10). Ninety-six-well polystyrene plates (Dynatech, Chantilly, Va.) were coated overnight at 4°C with 0.1-ml aliquots of 0.01 M carbonate buffer, pH 9.6, containing 3 µg of purified LOS or buffer alone (control wells). Wells were washed between steps and sequentially incubated with serial 10-fold dilutions of rat serum or 2-fold dilutions of human serum or anti-LOS antibodies (diluted in 1% bovine serum albumin [BSA]-PBS in duplicate) or 1% BSA-PBS alone, peroxidase-conjugated goat anti-rat or goat-anti human immunoglobulin (IgG, IgM, and IgA specific; Organon Teknika Corp., Cappel Research Products, Durham, N.C.), and substrate (*o*-phenylenediamine; Sigma). The OD (415 nm) of carbonate buffer wells was subtracted from the OD of LOS wells for each serum and dilution tested. The mean of duplicate determinations was calculated. Positive values were defined as ≥3 standard deviations above the mean absorbance of 1% BSA-PBS background controls. Assays were repeated at least twice on separate days.

**Immunoblot.** Purified LOS was resolved by SDS-urea-PAGE. After overnight incubation at 4°C in transfer buffer (0.03 M Tris, 0.19 M glycine, 20% methanol), LOS was electrophoretically transferred to nitrocellulose membranes (0.45-µm pore size; Schleicher & Schuell, Keene, N.H.) at a constant voltage of 70 V for 3 to 4 h. Membranes were blocked by overnight incubation at 4°C in PBS-0.5% gelatin-0.1% Tween 20 and sequentially incubated with rat (or human) sera, peroxidase-conjugated goat anti-rat (or human) immunoglobulin (IgG, IgM, and IgA specific; Cappel Research Products), and peroxidase substrate (4-chloro-1-naphthol). Antibody to outer membrane proteins was assessed following SDS-PAGE (11% gels) of a sarcosinate-insoluble cell envelope preparation followed by electrophoretic transfer of proteins to nitrocellulose and development as described for LOS.

**Bactericidal assay.** BPF-Hae was grown to mid-logarithmic phase, washed, and diluted to  $-2.0 \times 10^5$  CFU/ml in PBS containing 0.5 mM MgSO<sub>4</sub>, 0.1 mM CaCl<sub>2</sub>, and 1% BSA; 0.05 ml containing  $-10^4$  CFU was used in a total reaction mixture volume of 0.25 ml. Test sera were preheated to 56°C for 30 min to inactivate complement activity. A 0.05-ml volume of serum obtained from 7- to 10-day-old Sprague-Dawley infant rats was added as a source of complement in assays testing rat sera; infant rat sera contained no demonstrable bactericidal activity. For testing of human serum and human anti-LOS antibodies, a 60% concentration of newborn (precolostral) calf serum was used as a complement source. Aliquots of each reaction mixture (10 or, in some experiments, 20 µl) were plated in duplicate on chocolate agar plates before and after a 60-min 37°C incubation in a gyrotatory bath. The plates were incubated overnight at 37°C, and colonies were counted to determine the viable bacterial counts. The percentage killing of a test serum was calculated by comparison of the CFU after incubation with the test serum with either the CFU prior to incubation or the CFU after incubation of a reaction mixture without the test serum. The results represent the arithmetic means of duplicate or quadruplicate determinations. Bactericidal activity was defined as ≥50% killing after 60 min of incubation. The bactericidal titer was the highest dilution with bactericidal activity. Bactericidal assays were performed at least twice and on separate days.

**Infant rat protection assay.** The assay is described in

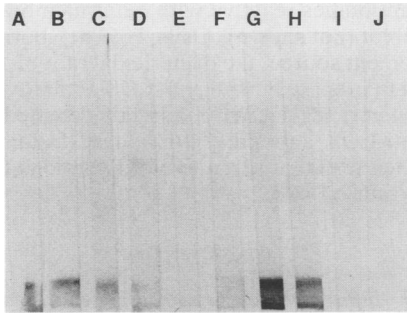


FIG. 1. Immunoblot assay to detect antibody to LOS. Purified LOS (5  $\mu$ g per lane) was subjected to SDS-urea-PAGE and transferred to nitrocellulose filters as described in Materials and Methods. Strips of nitrocellulose were blocked and sequentially exposed to serum (1:200), peroxidase-conjugated anti-rat immunoglobulin, and substrate as described in Materials and Methods. LOS migrated near the bottom of the gel. Lanes: A to C, anti-BPF-Hae (antiserum to whole bacterial cells); D to F, anti-LOS (obtained 1 week after three injections); G to I, anti-LOS (obtained after four injections); J, preimmune serum. Lanes A, D, G, and J were incubated with peroxidase-conjugated goat anti-rat IgG-, IgM-, and IgA-specific immunoglobulin; lanes B, E, and H were incubated with peroxidase-conjugated goat anti-rat IgG; lanes C, F, and I were incubated with peroxidase-conjugated goat anti-rat IgM.

reference 8. Natural litters of specific-pathogen-free 6-day-old Sprague-Dawley infant rats were obtained from Charles River Laboratories (Wilmington, Mass.). Litters of pups were pooled, and groups of 10 infant rats were reassigned to dams to minimize the effect of interlitter variation on the experimental results. Infant rats were passively immunized by subcutaneous injection of 0.1 ml of a 1:8 dilution of rat serum in PBS. Three hours later, rats received a bacterial challenge by intraperitoneal injection with  $\sim 10^5$  CFU of BPF-Hae in 0.1 ml of PBS containing 0.1% gelatin. Twenty-four hours later, bacteremia was quantified by plating 0.01 ml of blood from a tail vein on brain heart infusion agar plates with Levinthal's agar. The limit of detection of bacteremia was 100 CFU/ml of blood.

**Statistical analyses.** The magnitudes of bacteremia and mean percentages of killing were compared by using Student's two-tailed *t* test.

## RESULTS

**Characterization of anti-LOS sera.** The titers of anti-LOS antibody, as measured by EIA, were 100 for preimmunization rat sera and 10,000 for both preparations of anti-LOS antisera and for antiserum to whole bacterial cells. By immunoblot, anti-LOS antibody was detected in both preparations of anti-LOS antisera and in antiserum to whole bacterial cells (Fig. 1, lanes A, D, and G) but not in normal rat sera (lane J). As assessed by using IgG or IgM isotype-specific peroxidase conjugates, anti-LOS serum obtained 1 week after three injections contained predominantly IgM antibody (termed IgM anti-LOS serum; lane F, in which an IgM-specific conjugate was used, showed a more intense band than did lane E, in which an IgG-specific conjugate was used). Anti-LOS serum obtained later contained predominantly IgG antibody and no detectable IgM antibody (termed IgG anti-LOS serum; lane H, in which an IgG-specific conjugate was used, showed an intense band, and lane I, in which an IgM-specific conjugate was used, exhibited no visible band). In contrast to anti-whole cell antiserum,

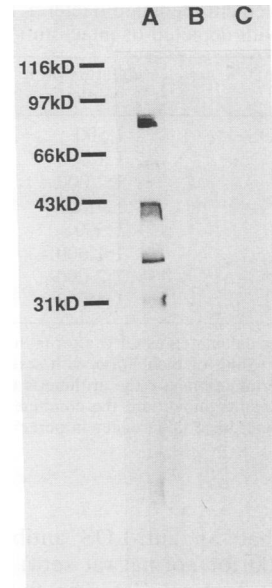


FIG. 2. Immunoblot assay to detect antibody to sarcosinate-insoluble cell envelope proteins (outer membrane proteins). A sarcosinate-insoluble outer membrane preparation of BPF-Hae F3037 was subjected to SDS-PAGE and electrophoretically transferred to nitrocellulose filters as described in Materials and Methods. Filters were blocked and sequentially incubated with rat sera diluted 1:100, peroxidase-conjugated goat anti-rat IgG-, IgM-, and IgA-specific immunoglobulin, and substrate. Lanes: A, anti-BPF-Hae (antiserum to whole bacterial cells); B, anti-LOS serum; C, preimmunization serum.

anti-LOS serum contained no detectable antibody to outer membrane protein as assessed by immunoblot (Fig. 2).

**Bactericidal activity of anti-LOS sera.** To determine bactericidal activity, sera were heated (56°C for 30 min) to deplete complement activity and assayed at a 20% concentration; 20% infant rat serum was included as a complement source. Results presented below are combined results of assays repeated five times on separate days. Percent killing (arithmetic mean of duplicate determinations of colony counts [CC]) was determined as CC before incubation - CC after 60 min of incubation/CC before incubation. Killing of <1% denotes >100% survival over the 60-min incubation, indicating a net multiplication of bacteria. Neither anti-LOS antiserum had bactericidal activity despite inclusion of a 20% concentration of antiserum (21%  $\pm$  28% and <1%  $\pm$  0% killing for IgM and IgG anti-LOS sera, respectively). (IgM anti-LOS serum exhibited some killing but less than 50% of the value for the inoculum.) These results contrasted with findings for antiserum to whole bacteria, which showed >99% kill with a 20% serum concentration and a bactericidal titer of 3,750.

Because it is possible that the LOS purification procedure resulted in a loss of epitopes on LOS, we studied the contribution of anti-LOS antibodies prepared against whole bacteria. We reasoned that immunization with whole bacterial cells presents the LOS in its native conformation in the outer membrane and should preserve epitopes of LOS which may be important for immunity. Antiserum to whole bacteria was depleted of anti-LOS antibody by using affinity chromatography; antiserum was incubated with a LOS affinity gel or sham gel to prepare antisera depleted of or with intact anti-LOS antibody, respectively. By EIA, anti-LOS-de-

TABLE 1. Complement-mediated bacterial killing by anti-whole cell serum with depleted or intact anti-LOS antibody

Rat serum	Dilution of serum	Mean % killing $\pm$ SD <sup>a</sup>
Depleted of anti-LOS	1:500	98.6 $\pm$ 0.7
	1:1,000	59 $\pm$ 28
	1:2,000	<1
	1:4,000	<1
Sham depleted	1:500	99.4 $\pm$ 0.6
	1:1,000	57 $\pm$ 32
	1:2,000	10 $\pm$ 9
	1:4,000	<1

<sup>a</sup> Percent killing was calculated as CFU after 60 min of incubation without serum - CFU after 60 min of incubation with serum/CFU after 60 min of incubation. CFU determinations are the arithmetic means of duplicate tubes plated in duplicate. Results shown are the combined results of experiments performed on 2 separate days. Differences in percent killing at each dilution were not significant.

pleted antisera had an anti-LOS antibody titer of  $\leq 100$  (versus 100 to 200 for normal rat sera) and sham-depleted antisera had a titer of  $>1,600$ . The two preparations had identical patterns of reactivity with outer membrane proteins by immunoblot (results not shown), suggesting that antibodies to outer membrane proteins were not removed by this procedure. The bactericidal titer was 1,000 for each preparation (Table 1), indicating that removal of anti-LOS antibodies did not result in a loss of bactericidal activity.

**Protective activity of anti-LOS sera.** The activity of anti-LOS antisera in protection of 6-day-old infant rats from BPF-Hae bacteremia is shown in Table 2. Neither IgG nor IgM anti-LOS serum decreased the incidence or magnitude of bacteremia. Antiserum against whole bacteria protected rats from BPF-Hae bacteremia.

**Bactericidal activity of human anti-LOS antibodies.** In an attempt to assess the relevance of these data to infection in humans, anti-LOS antibodies were purified from adult human serum by adsorption and subsequent elution from a LOS affinity gel; a control preparation was prepared by parallel experiments using adult human sera and a control gel. Anti-LOS activity was detectable by EIA to a dilution of 1:50 in the eluate from the LOS column; anti-LOS was not detected ( $<1:25$ ) in the eluate from the control column. Anti-LOS antibody was detectable in human serum to a dilution of 1:400. Anti-LOS antibody was detectable by immunoblot (against a proteinase K digest of a whole bacterial cell lysate) in the LOS column eluate but not in the control column eluate (not shown). Neither preparation

contained antibodies reactive with outer membrane proteins by immunoblot (not shown). Using 60% newborn calf serum as a complement source, the bactericidal titer of adult human serum used to prepare human anti-LOS antibody was 320. In contrast, bactericidal activity was not detected in affinity-purified anti-LOS antibodies (or eluate from the control column) when tested at serial twofold dilutions from 1:20 to 1:320, i.e., titer of  $<20$ .

## DISCUSSION

While *H. influenzae* biogroup *aegyptius* has long been recognized as an important cause of purulent conjunctivitis, BPF strains of the organism are unique in their ability to invade and survive in the intravascular compartment. Little is known about host factors which contribute to protection against BPF-Hae. In our model of experimental bacteremia, antiserum to whole BPF-Hae (containing both antibody to outer membrane proteins and LOS) has bactericidal activity and protective activity against experimental infection (8). Although this antiserum and antisera to purified LOS contained similar anti-LOS antibody titers, antisera to LOS had no (or minimal) bactericidal activity and were not protective against experimental bacteremia. Furthermore, depletion of anti-LOS antibodies from bactericidal antiserum to whole bacteria did not diminish its bactericidal activity. Thus, these data do not support a contribution of anti-LOS antibody to protection against experimental bacteremia with BPF-Hae. A difference in anti-LOS isotype distribution between nonprotective anti-LOS serum and protective anti-whole cell serum is unlikely to explain the absence of protection because we evaluated two antisera, one with predominantly IgM anti-LOS antibody and one with predominantly IgG anti-LOS antibody, and each lacked detectable protective activity. Furthermore, we were unable to demonstrate bactericidal activity by using anti-LOS antibodies purified from a human serum which contained bactericidal activity. These data support the hypothesis that anti-LOS antibodies may not be important for prevention of bacteremia with BPF-Hae.

There are limitations to the conclusion that antibody to LOS does not contribute to protection against BPF. It is possible that the LOS purification process resulted in a loss of LOS epitopes which stimulate production of bactericidal and protective antibodies. Furthermore, purified LOS bound to gel was used to deplete anti-LOS antibodies from anti-whole cell serum; this procedure could possibly have allowed putative bactericidal anti-LOS antibodies to remain in the antiserum. Because purified LOS was used in the EIA to measure anti-LOS antibodies, putative protective anti-LOS antibodies might be present in anti-whole cell sera and would not have been detected by EIA. In addition, we studied only a single BPF-Hae isolate, and several different LOS phenotypes have been described among Brazilian isolates of BPF-Hae (1). We purified anti-LOS antibodies from only a single human serum, and the resultant material had a lower LOS antibody concentration than did the original serum. It is possible that anti-LOS antibodies purified from a patient with BPF contain a higher anti-LOS antibody titer and have demonstrable bactericidal activity. Finally, even if anti-LOS antibodies have no effect on bacterial clearance, anti-LOS antibody might partially ameliorate the severity of BPF by binding to endotoxin. Such an effect would not be noted in the infant rat model of bacteremia because infected rats do not show clinical evidence of disease (8).

LOS of *H. influenzae* may undergo spontaneous LOS

TABLE 2. Protective activity of passively administered rat antisera against experimental bacteremia with BPF-Hae

Rat serum <sup>a</sup>	Incidence of bacteremia		Geometric mean magnitude of bacteremia (CFU/ml $\pm$ SD)
	No. with bacteremia/total	%	
Anti-whole cell	0/10		$<10^{2.0} \pm 0$
IgM anti-LOS	9/10	90	$10^{3.0} \pm 2.7^b$
IgG anti-LOS	9/9	100	$10^{3.0} \pm 2.8^b$
Preimmunization	9/10	90	$10^{3.4} \pm 3.5^b$

<sup>a</sup> A 0.1-ml sample of a 1:8 dilution was injected subcutaneously 3 h prior to intraperitoneal injection with  $10^5$  CFU of logarithmic-phase bacteria.

<sup>b</sup> Not significantly different from each other;  $P < 0.05$  compared with the value for anti-whole cell (two-tailed Student *t* test).

antigenic variation (phase variation) resulting in a loss or acquisition of oligosaccharide moieties and a change in reactivity with anti-LOS antibodies (12). LOS phase variation occurring *in vivo* could result in a difference between the LOS phenotype of inoculated bacteria and the phenotype of bacteria recovered from the blood, which could result in a failure of an anti-LOS antibody directed at LOS antigens of the inoculated bacteria to protect. We have observed differences in LOS phenotype between the original and animal-passaged BPF-Hae F3031 (as assessed by electrophoretic mobility by SDS-urea-PAGE and reactivity with a panel of monoclonal anti-LOS antibodies [7a, 9]). However, it is unlikely that this observation confounds interpretation of these results because the same LOS phenotype (the animal-passaged variant) was used for all aspects of this study: for preparation of purified LOS, for rat immunization, as an antigen for EIA and immunoblot, for bactericidal assays, and for experimental infection in infant rats. As assessed by a reactivity panel of monoclonal antibodies against LOS, the animal-passaged phenotype maintained identical reactivity patterns after one or multiple animal or *in vitro* passages (7a).

Several investigators have examined the bactericidal and protective activities of anti-LOS antibodies against a related pathogen, *H. influenzae* type b (Hib). In studies similar to ours, Shenep et al. (11) found that polyclonal rabbit antiserum against LOS of Hib did not protect against experimental infection in infant rats. In addition, Shenep et al. (10) showed that children with Hib meningitis had high titers of antibody to LOS of Hib at the time of presentation of meningitis, suggesting that antibody to LOS does not contribute to protection against development of Hib infection in humans. In contrast, Gulig and Hansen (3) developed a monoclonal antibody against 39-kDa protein-associated LOS of Hib which had both bactericidal and protective activities in an infant rat model of experimental infection. Marks et al. (6) found that polyclonal rabbit antiserum against *E. coli* J5, a mutant strain with only core lipopolysaccharide, reduced the mortality of mice following intraperitoneal injection with Hib. Thus, the role of anti-LOS antibody in protection against Hib remains unclear.

Finally, we have found the anti-LOS antiserum described in this study, which contains predominantly IgG anti-LOS antibody, blocks the bactericidal activity of antiserum to whole bacteria and to outer membrane proteins. In addition, anti-LOS antiserum diminished the protective activity of anti-outer membrane protein antiserum (7). Thus, rather than contributing to protection against BPF, it is possible that certain anti-LOS antibodies interfere with bacterial killing, thereby contributing to the development of this disease.

#### ACKNOWLEDGMENTS

This study was supported in part by the Long Island Jewish Medical Center Faculty Research Awards Program. Vicki B. Peters

was the recipient of a fellowship award from the Long Island Jewish Medical Center Research Awards Program.

We thank Atqia Rizvi for technical assistance, the BPF Study Group for providing and characterizing the strain used in this study, Brad Fenwick for supplying precolostral newborn calf sera, and George Carlone, Linda Gheesling, and Alice Erwin for helpful advice concerning LOS purification and characterization.

#### REFERENCES

1. Carlone, G. M., L. Gorelkin, L. L. Gheesling, A. L. Erwin, S. K. Hoiseth, M. H. Mulks, S. P. O'Connor, R. S. Weyant, J. Murick, L. Rubin, R. S. Munford III, E. H. White, R. J. Arko, B. Swaminathan, L. M. Graves, L. W. Mayer, M. K. Robinson, S. P. Caudill, and the Brazilian Purpuric Fever Study Group. 1989. Potential virulence-associated factors in Brazilian purpuric fever. *J. Clin. Microbiol.* **27**:609-614.
2. Erwin, A. L., R. S. Munford, and the Brazilian Purpuric Fever Study Group. 1989. Comparison of lipopolysaccharides from Brazilian purpuric fever isolates and conjunctivitis isolates of *Haemophilus influenzae* biogroup aegyptius. *J. Clin. Microbiol.* **27**:762-767.
3. Gulig, P. A., and E. J. Hansen. 1985. Coprecipitation of lipopolysaccharide and the 39,000-molecular-weight outer membrane protein of *Haemophilus influenzae* type b by lipopolysaccharide-directed monoclonal antibody. *Infect. Immun.* **49**:819-827.
4. Harrison, L. H., G. A. daSilva, M. Pittman, D. W. Fleming, A. Vranjac, C. V. Broome, and the Brazilian Purpuric Fever Study Group. 1989. Epidemiology and clinical spectrum of Brazilian purpuric fever. *J. Clin. Microbiol.* **27**:599-604.
5. Hitchcock, P. J., and T. M. Brown. 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels. *J. Bacteriol.* **154**:269-277.
6. Marks, M. I., E. J. Ziegler, H. Douglas, L. B. Corbeil, and A. I. Braude. 1982. Induction of immunity against lethal *Haemophilus influenzae* type b infection by *Escherichia coli* core lipopolysaccharide. *J. Clin. Invest.* **69**:742-749.
7. Peters, V. B., L. G. Rubin, and the BPF Study Group. 1990. Anti-lipooligosaccharide antibody blocks protective activity of antiserum in experimental bacteremia due to a Brazilian purpuric fever isolate of *Haemophilus influenzae* biogroup aegyptius. *Pediatr. Res.* **27**:179A.
- 7a. Rubin, L. Unpublished data.
8. Rubin, L. G., E. S. Gloster, G. M. Carlone, and the BPF Study Group. 1989. An infant rat model of bacteremia with Brazilian purpuric fever isolates of *Haemophilus influenzae* biogroup aegyptius. *J. Infect. Dis.* **160**:476-482.
9. Rubin, L. G., J. St. Geme, and the BPF Study Group. 1991. Role of pili and lipooligosaccharide phenotypes in the virulence of a Brazilian purpuric fever strain of *Haemophilus influenzae* biogroup aegyptius. *Pediatr. Res.* **29**:183A.
10. Shenep, J., R. Munson, and D. Granoff. 1982. Human antibody responses to lipopolysaccharide after meningitis due to *Haemophilus influenzae* type b. *J. Infect. Dis.* **145**:181-190.
11. Shenep, J., R. S. Munson, Jr., S. J. Barenkamp, and D. M. Granoff. 1983. Further studies of the role of noncapsular antibody in protection against experimental *Haemophilus influenzae* type b bacteremia. *Infect. Immun.* **42**:257-263.
12. Weiser, J. N., A. A. Lindberg, E. J. Manning, E. J. Hansen, and E. R. Moxon. 1989. Identification of a chromosomal locus for expression of lipopolysaccharide epitopes in *Haemophilus influenzae*. *Infect. Immun.* **57**:3045-3052.